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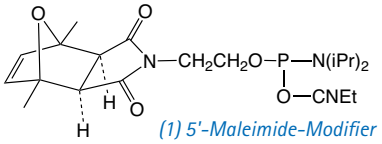
TECHNICAL BULLETIN - 5'-MALEIMIDE MODIFIER PHOSPHORAMIDITE

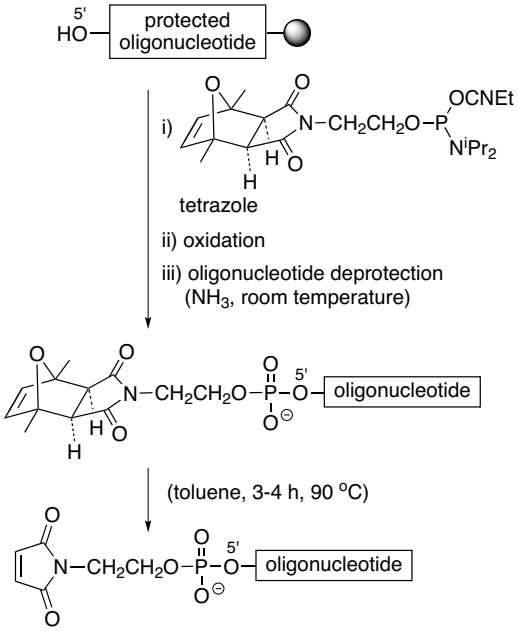
5'-Maleimide Modifier Phosphoramidite (1), developed at the University of Barcelona, incorporates a maleimide cycloadduct that is stable to ammonium hydroxide at room temperature. This phosphoramidite can be incorporated into DNA and RNA with both phosphate and phosphorothioate linkages.[1,2] A retro Diels-Alder reaction deprotects the maleimide immediately prior to conjugation, as shown in Figure 2. See The Glen Report 23.2, p6 for further details.

FIGURE 1: 5'-MALEIMIDE MODIFIER



(1) 5'-Maleimide-Modifier

FIGURE 2: INCORPORATION OF 5'-MALEIMIDE-MODIFIER



SYNTHESIS

We recommend a 3 minute coupling time. No other changes are needed from standard method recommended by the synthesizer manufacturer.

OLIGONUCLEOTIDE DEPROTECTION

Room temperature deprotection is recommended to avoid degradation of the protected maleimide. This is accomplished using UltraMild phosphoramidites which require 2 hours with ammonium hydroxide or 4 hours with potassium carbonate in methanol. Standard phosphoramidites (Bz-dA, dmf-dG, ibu-dG, and Ac-dC) can be used with AMA deprotection (Ammonium hydroxide, 40% Methylamine in water (1:1), 2 hours, room temperature). See The Glen Report on Deprotection for additional information: http://www.glenresearch.com/Technical/Deprotection.pdf.

MALEIMIDE DEPROTECTION (RETRO DIELS-ALDER)

The deprotection of the [protected maleimide] oligonucleotide is a retro Diels-Alder reaction that generates the reactive maleimide. Two procedures have been described for the retro Diels-Alder reaction. The microwave irradiation procedure is effective but requires specialized equipment. The toluene procedure can be carried out in any lab without the need for specialized equipment.

MICROWAVE-PROMOTED DEPROTECTION

A solution (500-1000 µL) of the [protected maleimide] oligonucleotide in a 1:1 (v/v) MeOH/H_2O mixture (25 µM concentration) was introduced in a microwave vial and irradiated for 90 min at 90 °C. The solvent was removed under vacuum to give the maleimide-modified oligonucleotide.

DEPROTECTION BY HEATING IN TOLUENE

The oligo (aqueous solution) is first taken to dryness or lyophilized, and dried by co-evaporation with anhydrous acetonitrile and then three co-evaporations with anhydrous toluene. After drying, the oligo is suspended in 2mL anhydrous toluene and the Retro Diels-Alder reaction is completed at 90°C for four hours. The Retro Diels-Alder reaction requires anhydrous conditions and any significant level of moisture can cause incomplete deprotection, hydrolysis, and/or addition of water to the maleimide. The evaporation of the toluene leaves a white residue ready for conjugation. The procedure is described in detail below.

SAMPLE PROCEDURE FOR MALEIMIDE DEPROTECTION

This procedure worked well for a variety of oligos.

Materials

Anhydrous acetonitrile, Glen Research, (40-4050-50)
Anhydrous toluene, dried with activated 4Å molecular sieves
Gas tight syringe, 2.5mL, with Luer adapter, Hamilton
4mL screw cap vial, Wheaton (224882) (see Photo 1)
4mL screw cap with hole, Glen Research (Empty Twist column, 20-0300-00) (see Photo 1)
11mm septum, Restek (27162) (see Photo 1)
16-gauge needle, 1-1/2 inch, clipped and filed flat, Becton Dickinson (305198) (see Photo 1)
20 gauge needle, 1-1/2 inch, Becton Dickinson (305176) (see Photo 1)
Rotary evaporator with water bath at 40°C or below
Lyophilization flask, 75mL, Virtis (312900)
14/20 adapter. Ace Glass (6704-04)

Procedure for Maleimide Deprotection

1. Aliquot 50 nmoles of desalted or purified oligo to a clean 4mL screw cap vial.
2. Remove water on a vacuum concentrator.
3. Add 2mL anhydrous acetonitrile. The oligo will not dissolve in the acetonitrile.
4. Attach the septum and the screw cap with hole.
5. Pierce the center of the septum with a needle at a slight angle so the needle will align straight after piercing. Insert 16 gauge needle.
6. Place the vial in a lyophilization flask on a rotary evaporator and evaporate the acetonitrile. Avoid bumping by gently increasing the vacuum. Evaporation should be complete in about 20 minutes.
7. Back fill the rotary evaporator with dry argon to release the vacuum.
8. Rinse the 2.5mL syringe and 20-gauge needle with 2mL anhydrous toluene and then use the syringe to add 2mL of anhydrous toluene to the vial. The 20-gauge needle should slide through the 16-gauge needle (Photo 2) without the need to entirely remove the vial from the flask.
9. Evaporate the toluene, gently increasing the vacuum to avoid bumping. Use a water bath with a temperature of 37°C. A white residue should be visible at the bottom of the vial. Evaporation should be complete in about 20 minutes. See Photo 3.
10. Repeat twice more, back-filling the rotary evaporator with dry argon when releasing the vacuum.
11. Remove the flask from rotary evaporator.
12. Add 2mL of toluene and remove the 16-gauge needle.
13. Place the vial in a heat block at 90°C for 4 hours.
14. After cooling to room temperature, insert the 16-gauge needle and evaporate the toluene in the rotary evaporator.
15. The oligo is now ready for conjugation.

Results

Figure 3 shows the RP HPLC of a [protected maleimide] oligo. Figure 4 shows the maleimide oligo after retro Diels-Alder reaction in toluene at 90°C. Figure 5 shows the oligo after the maleimide is conjugated with cysteine.

CONJUGATION OF MALEIMIDE WITH THIOLS

The conjugation reaction occurs in aqueous buffer solution under mild conditions and is completed in 30-60 minutes with a final oligo concentration of 80 µM and 5-10 equivalents of thiol compound. We recommend a pH range of 6.5 -7.5. We used a sodium phosphate buffer at pH 6.5 successfully in our work. The group in Barcelona carried out their conjugations successfully using a triethylammonium acetate buffer at pH 7.8. Clearly, a pH range of 6.5 to 7.8 in your buffer of choice would be acceptable.

PHOTO 1: VIAL, CAP AND NEEDLES

PHOTO 2: ASSEMBLED VIAL

PHOTO 3: VIAL IN ROTARY EVAPORATOR

REFERENCES

1. A. Sánchez, E. Pedroso, A. Grandas, *Org. Lett.* 2011, **13**, 4364-4367.
2. A. Sanchez, E. Pedroso, and A. Grandas, *Bioconjug Chem*, 2012, **23**, 300-7.

FIGURE 3: [PROTECTED MALEIMIDE] OLIGO

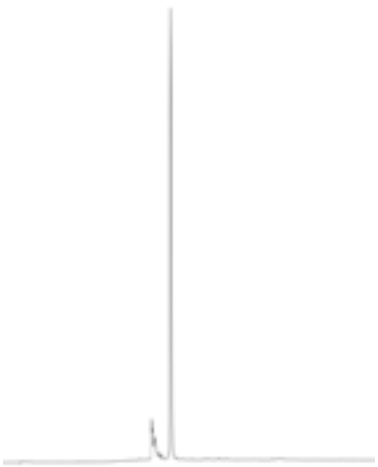


FIGURE 4: DEPROTECTED MALEIMIDE OLIGO

FIGURE 5: MALEIMIDE OLIGO + CYSTEINE